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Mapping of quantitative trait loci affecting resistance/susceptibility to *Sarcocystis miescheriana* in swine

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Abstract

The outcome of infectious diseases in vertebrates is under genetic control at least to some extent. In swine, e.g., marked differences in resistance/susceptibility to *Sarcocystis miescheriana* have been shown between Chinese Meishan and European Pietrain pigs, and these differences are associated with high heritabilities. A first step toward the identification of genes and polymorphisms causal for these differences may be the mapping of quantitative trait loci (QTLs). Considering clinical, immunological, and parasitological traits in the above model system, this survey represents the first QTL study on parasite resistance in pigs. QTL mapping was performed in 139 F_2 pigs of a Meishan/Pietrain family infected with *S. miescheriana*. Fourteen genome-wide significant QTLs were mapped to several chromosomal areas. Among others, major QTLs were identified for bradyzoite numbers in skeletal muscles ($F = 17.4$; $p < 0.001$) and for *S. miescheriana*-specific plasma IgG_2 levels determined 42 days p.i. ($F = 20.9$; $p < 0.001$). The QTLs were mapped to different regions of chromosome 7, i.e., to the region of the major histocompatibility complex (bradyzoites) and to an immunoglobulin heavy chain cluster, respectively. These results provide evidence for a direct and causal role for gene variants within these gene clusters (*cis*-acting) in differences in resistance to *S. miescheriana*.

Keywords: Disease resistance; Sarcocystis; Swine; Quantitative trait loci

The outcome of infectious diseases in vertebrates is under genetic control at least to some extent [1], but the gene loci contributing to such phenotypic variations of the host are unknown in most cases. Linkage mapping using dense genetic maps is a straightforward approach for locating genes that control inherited diseases monogenetically or quantitatively. Quantitative trait locus (QTL) mapping may be the first step toward the identification of relevant genes and causal polymorphisms for important traits [2]. Using alleles of marker loci segregating within families, QTL analysis correlates phenotypic variation with chromosomal sections throughout the entire genome. QTL analysis provides insight into the chromosomal localization of effects (gene loci), such as those associated with disease resistance, and thus helps in defining candidate genes. It also provides insight into the individual contribution of these loci, provided that the trait's variation is based on many QTLs with small effects or few QTLs with large effects.

Concerning the resistance/susceptibility of farm animals to parasitic infections, QTLs have been mapped for trypanotolerance in cattle [3], coccidia resistance in poultry [4], and intestinal nematode resistance in sheep [5–7]. In the pig, approximately 1700 QTLs have been mapped for more than 280 traits but mainly for fattening performance, carcass and meat quality, and reproduction [8,9]. The only QTL study on the resistance/susceptibility to an infectious agent, i.e., the pseudorabies virus, was reported by Reiner et al. [10].

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We have recently developed a parasitological model system in pigs that seems suitable for mapping QTLs for a parasitic disease; it uses *Sarcocystis miescheriana*, a common protozoan parasite, in swine [11,12]. Cyst-forming coccidia of the genus *Sarcocystis* are among the most prevalent parasites of livestock [13], and *S. miescheriana* represents one of three species using the pig as the intermediate host. Tissue cysts of *Sarcocystis* spp. have been isolated from 8–36% of slaughter sows in various countries, with seroprevalences ranging between 15 and 69% [14]. The clinical and pathological outcomes of the disease are closely linked with the numbers of sporocysts ingested, making the parasite an ideal controllable model for the study of porcine–protozoan interaction [15].

The life cycle of *S. miescheriana* includes two merogonies in the intermediate host and a gamogony in the final host, which excretes infectious, sporulated sporocysts [16]. After oral uptake and ingestion of the sporocysts by the pig, the sporocysts develop a first and a second generation of merozoites with schizogonies on days 5–7 and 12–14, respectively [17]. Merozoites of the second stage penetrate striated muscle fibers and cardiac muscle cells. Cyst formation starts 27 days p.i. [18,19].

The present paper applies the above model with *S. miescheriana* to a Pietrain/Meishan F_2 family. This is the first QTL study on parasite resistance in pigs.

Results

Phenotype

The phenotype of the F_2 pigs was defined by clinical and clinical–chemical traits, specific antibody levels, and the number of bradyzoites per gram of musculus longissimus dorsi (Mld) and cardiac muscle (Table 1). The details, including genetic parameters of these traits, have been described elsewhere [12]. The F_2 pigs showed fever on days 12–14 p.i., i.e., at the time of the second schizogony. During the chronic stage of the infection (represented by day 42), the mean body temperature remained at physiological levels, but the variance of the data was increased. Changes in clinical findings were accompanied by changes in hematological traits. During the acute stage of the infection, platelet counts decreased significantly. The numbers of eosinophils increased after infection and remained at an elevated level until necropsy. Aspartate amino transferase (AST) and creatine kinase (CK) levels increased significantly throughout the observation period.

Specific antibody levels increased throughout the whole observation period and showed remarkable variation between F_2 pigs. The IgM response was relatively weak, whereas distinctly enhanced IgG levels were detected 12 days p.i. and later. The number of bradyzoites found 70 days p.i. was highly variable, ranging from 0 to 1.1×10^6/g in the cardiac muscle and from 0 to 7.8×10^6/g in the Mld.

QTL

QTL analysis provided 42 chromosome-wide significant (putative) QTLs and 14 genome-wide significant QTLs. Major QTLs were found on chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 17, and X (Table 2). The F values for genome-wide significance thresholds were estimated as approximately 14.0 ($p \leq 0.001$), 11.2 ($p \leq 0.01$), and 8.2 ($p \leq 0.05$), respectively. The F threshold for a chromosome-wide significance resembled 5.5 ($p \leq 0.05$). Results of the QTL analysis, including all putative QTLs (significant on a 5% chromosome-wide level with $F \geq 5.5$), are shown in Table 2. Positive a values indicate that Pietrain alleles result in higher values than Meishan alleles, and vice versa. A detailed illustration of the allelic effects of the peak markers of the five major QTLs is given in Fig. 2.

Three QTLs for bradyzoite numbers mapped to SSC7 (Mld; $p < 0.001$), SSC16 (Mld), and SSC2 (cardiac muscle). The QTLs on SSC7 and SSC16 together explained 44.7% of the F_2 phenotypic variance of this trait. The QTL on SSC7 mapped directly to the region of the MHC (Fig. 1). In all cases, alleles of the Pietrain breed increased bradyzoite numbers (Table 2, Fig. 2).

Seven putative and four genome-wide significant QTLs were mapped for antibody titers. QTLs for IgM and IgG mapped to SSC5, SSC12, and SSC13. Genome-wide significant QTLs mapped to SSC4 (IgG_1, day 42; $p < 0.01$), SSC7 (IgG_2, day 42;

Table 1
Response of Meishan/Pietrain F_2 pigs to an experimental infection with 50,000 sporocysts of *Sarcocystis miescheriana*

Trait	Day 0	Day 14	Day 28	Day 42
Parasite burden				(Day 70)
Bradyzoite counts in the heart (log No./g)				2.84 ± 1.99
Bradyzoite counts in the Mld (log No./g)				4.26 ± 2.04
Specific antibodies				
IgM (OD)	0.25 ± 0.08	0.30 ± 0.11	0.43 ± 0.13	0.49 ± 0.14
IgG (OD)	0.037 ± 0.06	0.043 ± 0.08	0.141 ± 0.16	0.354 ± 0.23
IgG_1 (OD)	0.021 ± 0.09	0.024 ± 0.10	0.048 ± 0.12	0.102 ± 0.25
IgG_2 (OD)	0.055 ± 0.13	0.066 ± 0.14	0.142 ± 0.23	0.234 ± 0.29
Clinical trait: rectal temperature (°C)	39.25 ± 0.20	39.70 ± 0.50	39.19 ± 0.41	39.18 ± 0.46
Clinical–chemical traits				
Platelets (G/l)	422.09 ± 121.25	280.10 ± 100.46	344.12 ± 117.36	404.80 ± 113.62
Eosinophils (%)	1.97 ± 1.57	3.83 ± 2.79	3.49 ± 3.41	3.43 ± 2.31
AST (IU/L)	22.49 ± 7.58	28.67 ± 12.55	35.18 ± 14.46	80.56 ± 36.92
Log CK (log IU/L)	2.32 ± 0.35	2.36 ± 0.43	2.53 ± 0.33	3.17 ± 0.29

The data represent arithmetical means ± standard deviations.

Table 2
QTLs sorted by chromosome and *F* value

SSC	cM	*F*	*p*	Marker range		Trait	Time	% *s*	*a*	±SD$_a$	*d*	±SD$_d$
1	106	9.61	*	Sw803	Sw705	CK/AST	28	14.87	5.91	1.81	−7.82	2.98
1	76	7.27		Sw780	Sw803	AST	42	8.59	−0.01	0.02	−0.13	0.03
1	113	6.07		Sw705		Eosinophils	14	9.78	0.61	0.28	−1.00	0.40
1	72	5.90		Sw780		Log CK	42	10.51	0.01	0.04	−0.17	0.05
2	100	10.42	*	Sw803	Sw705	Rectal temperature	14	15.689	−0.039	0.04	0.02	0.14
2	121	6.07		Sw705		Rectal temperature	14	9.71	−0.01	0.06	−0.33	0.09
2	87	6.02		Sw803		Rectal temperature	28	9.84	−0.04	0.05	0.26	0.08
2	64	5.68		S0091		Bradyzoites heart	70	13.80	0.30	0.17	−0.58	0.22
3	42	9.12	*	Swr978		Rectal temperature	42	14.57	0.19	0.05	−0.15	0.08
3	26	7.93		S0206		Platelets	42	14.06	−42.39	17.02	72.66	23.55
3	26	7.93		S0206		Platelets	42	14.06	−42.39	17.02	72.66	23.55
3	36	7.11		Swr978		Platelets	42	12.13	−56.66	15.09	−6.74	20.98
3	31	6.52		S0206	Swr978	Log CK	0	11.50	0.15	0.04	0.01	0.06
3	25	6.29		S0206		Platelets	0	9.93	−5.50	15.72	−75.06	21.28
4	32	8.87	*	Sw853	S0001	IgG1	42	15.04	0.10	0.04	−0.16	0.05
5	23	7.60		Sw413	Sw1482	Platelets	14	15.63	−27.72	14.94	−88.57	25.56
5	96	6.51		S0018		IgG	28	11.22	−0.057	0.02	−0.08	0.03
5	118	6.41		S0018	Sw378	IgG2	28	11.232	0.081	0.031	−0.14	0.06
5	30	5.57		Sw1482		Platelets	42	9.76	−31.72	15.12	−60.14	23.06
5	50	5.55		Swr453		IgM	0	9.45	−0.03	0.01	0.0049	0.013
6	68	8.66	*	Sw492	Sw824	CK/AST	42	13.71	−6.15	1.49	−0.17	2.32
6	158	7.06		Sw322	Sw2052	Rectal temperature	42	11.57	−0.098	0.06	−0.297	0.083
6	84	6.74		Sw492	Sw824	Platelets	14	14.12	−33.82	21.05	128.24	43.35
6	86	6.71		Sw492	Sw824	Platelets	42	11.53	−35.07	24.37	143.62	47.36
6	60	6.45		S0087	Sw492	Rectal temperature	14	10.34	0.21	0.10	0.51	0.16
6	60	6.13		Sw492		CK/AST	0	10.11	−3.30	2.14	−11.34	3.34
7	152	20.92	***	Sw764		IgG2	42	29.04	−0.21	0.03	−0.06	0.05
7	57	17.39	***	LTA	Sw2428	Bradyzoites Mld	70	32.88	0.69	0.12	−0.08	0.19
7	27	5.95		S0064		Platelets	14	12.68	−18.60	14.24	56.18	19.18
7	137	5.64		S0212		Log CK	42	10.13	−0.09	0.03	−0.08	0.05
8	124	9.30	*	Sw790	Sw1980	CK/AST	42	14.57	−1.20	1.49	−10.16	2.37
8	91	6.59		Sw2160	Sw790	IgG2	28	11.59	0.07	0.03	−0.09	0.04
10	87	9.65	*	Sw1708	Sw2067	AST	42	16.02	−0.10	0.02	0.05	0.04
10	0	5.82		Sw767		CK/AST	42	9.64	−2.18	1.21	−4.73	1.64
11	70	6.02		S0182	Sw903	Rectal temperature	14	9.75	−0.06	0.08	0.43	0.13
11	69	5.66		S0182	Sw903	Eosinophils	14	9.24	−1.05	0.31	−0.18	0.54
12	27	6.46		Sw959		IgG	42	11.22	−0.10	0.03	0.05	0.04
12	107	6.26		Swc23		IgM	14	10.59	−0.03	0.01	−0.03	0.02
12	0	5.97		S0143		Eosinophils	28	9.90	1.06	0.41	−1.08	0.59
12	0	5.62		S0143		Eosinophils	28	9.68	1.02	0.43	−1.21	0.63
13	25	7.22		S0219	Sw344	Rectal temperature	0	11.28	0.03	0.03	−0.19	0.05
13	18	6.93		S0219	Sw344	IgM	14	11.79	−0.04	0.01	−0.05	0.02
14	33	8.04		Sw2519	Sw761	AST	14	13.84	−0.08	0.02	0.01	0.03
14	0	6.86		Sw2038		Log CK	42	12.05	0.13	0.03	−0.01	0.05
14	0	5.71		Sw2038		CK/AST	0	9.48	3.92	1.56	−5.49	2.22
15	56	5.97		Sw2083	Sw1983	Rectal temperature	14	9.61	0.18	0.06	−0.17	0.10
16	37	7.46		Sw1809	Swr2480	Rectal temperature	14	11.68	0.31	0.08	0.06	0.13
16	15	6.20		Sw419		Bradyzoites Mld	70	11.67	0.14	0.12	−0.06	0.19
17	83	15.61	***	Sw1031	Sw2431	IgG2	42	23.36	0.18	0.05	−0.35	0.07
17	22	13.02	**	Sw335	S0296	Eosinophils	28	19.24	1.95	0.48	−2.48	0.74
17	41	8.32	*	S0296	Sw1031	Eosinophils	42	13.28	1.07	0.34	−1.72	0.53
17	26	6.42		Sw335	S0296	Eosinophils	28	10.84	1.28	0.51	−2.11	0.74
18	37	7.31		Sw787	S0120	Platelets	14	15.12	43.02	13.31	47.14	21.79
X	105	15.02	***	Sw1608		IgG2	28	23.19	0.19	0.05	−0.73	0.15
X	31	9.38	*	Sw949	Sw2126	Eosinophils	42	15.19	−2.40	0.65	4.06	1.23
X	126	6.79		Sw1608	Sw2588	Eosinophils	28	11.10	0.74	1.25	15.32	4.78

SSC, chromosome number; cM, position on the chromosome; *F*, *F* value; *p*, level of significance; marker range, approximate location of the QTL relative to markers; trait and time, days postinfection when the trait was measured; %s, percentage of phenotypic variance explained by the QTL; *a* and *d*, additive and dominance effects of the QTL; ±SD$_a$, ±SD$_d$, standard deviations of the *a* and *d* values. All QTLs are significant at least on a $p \leq 0.05$ chromosome-wide level, genome-wide significant QTLs are noted: *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$.

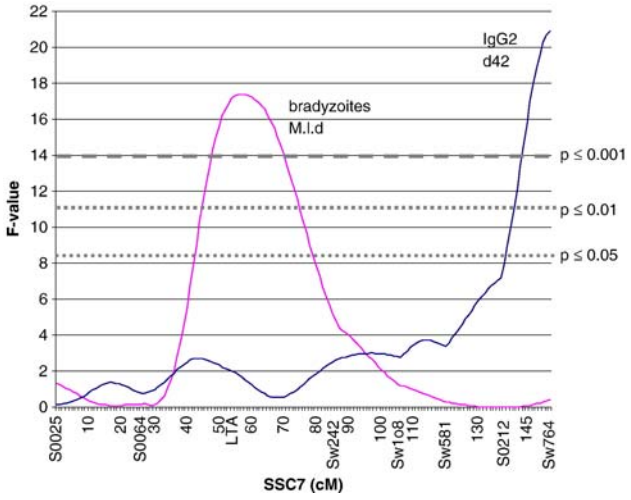

Fig. 1. Major QTLs on SSC7 associated with bradyzoite numbers per gram of Mld on day 70 p.i. and IgG_2 titers on day 42 after experimental infection with 50,000 sporocysts of *S. miescheriana*. The QTLs peak at the positions of the MHC/TNF (bradyzoites) and an immunoglobulin heavy-chain gene cluster (IgG_2 titers). Both QTLs are highly significant on a genome-wide level.

$p < 0.001$; Table 2, Figs. 1 and 2), SSC17 (IgG_2, day 42; $p < 0.001$), and SSCX (IgG_2, day 28; $p < 0.001$). Together, the QTLs for IgG_2 on day 42 explained 52.4% of the F_2 phenotypic variance.

Ten QTLs were found for body temperature, most of which were putative. One QTL on SSC2 (body temperature during acute infection) and a second QTL on SSC3 (body temperature during chronic infection) were significant on a genome-wide level.

AST and CK levels were associated with 11 QTLs, mainly during the chronic stage of the disease. A genome-wide significant QTL mapped to SSC10 (AST). Platelet numbers were under the control of 4 QTLs on SSC5, SSC6, SSC7, and SSC18 during acute infection and 3 QTLs on SSC3, SSC5, and SSC7 during the chronic stage of disease. Eosinophils were linked with 4 QTLs on SSC1, SSC11, SSC12, and SSC17 (Fig. 2C) during the acute and chronic stages of the disease.

Significant genome-wide single QTLs explained between 13.7 and 32.9% of the total F_2 phenotypic variance. The F_2 variance explained by putative QTLs ($F > 5.5$) varied between 8.6 and 15.6%. The traits with the highest ratios of explained variance were bradyzoite numbers in Mld (32.9%), IgG_2 titers (day 42; 29.0 and 23.4%), and eosinophils (day 28; 19.2%). QTL effects were particularly high at the cyst-forming stage of the disease, but generally, the effects were remarkable also during the acute phase. The cumulative F_2 variance ranged between 5.2 and 69.8% for individual traits (data not shown). The highest ratios of cumulative variance were explained by the body temperature during the acute stage of disease (day 14; 69.3%), followed by platelet numbers (day 14; 57.6%), IgG_2 levels (day 42; 52.4%), eosinophils (day 28; 49.7%), and bradyzoites in the Mld (44.6%). The numbers of bradyzoites and eosinophils and the IgG_2 titers were under the control of two QTLs each, while body temperature was controlled by six QTLs, each with smaller effects.

Discussion

A recent study by Reiner et al. [11] described remarkable, breed-dependent differences in resistance/susceptibility to *S. miescheriana* infection in swine: experimentally infected Chinese Meishan and European Pietrain pigs differed by 3 to 6 standard deviations in clinical and parasitological traits. Moderate to high heritabilities found in a Meishan/Pietrain F_2 family for bradyzoite numbers, IgM and IgG_2 levels, and some clinical and clinical–chemical traits suggested that distinct allelic variants of gene loci (QTL) are involved in the variability of the traits [12]. Based on these data, the present study maps QTLs associated with resistance/susceptibility in porcine sarcocystosis.

The major difference between the two breeds of pigs in response to *S. miescheriana* concerned the number of bradyzoites that developed in the musculature, which was 20 times higher in the Pietrain pigs than in the Meishan pigs. The main QTL for bradyzoite number in Mld mapped to the marker interval LTA (lymphotoxin A)–Sw2428, a region containing gene clusters of the major histocompatibility complex (MHC) and TNF. Associations of MHC variability with disease susceptibility are well documented in vertebrates [20]. An example in pigs concerns the response to *Trichinella spiralis* [21]. TNF is a predominantly proinflammatory cytokine, which mediates many immune functions and the growth of various cell types [21–23]. For example, it plays a crucial role in controlling the related parasite *Toxoplasma gondii* in mice [24]. The power of this QTL was well correlated with the heritability of 0.68 for the trait [12]. A second QTL for bradyzoite numbers mapped to the region of Sw419 on SSC16 ($F = 6.2$). Both QTLs together explained 44.6% of the F_2 phenotypic variance of this trait. Pietrain alleles increased bradyzoite numbers in both cases.

The most prominent effect of the present study was mapped to SSC7, on the telomere of the long arm of the chromosome (Sw764). This QTL with an F value of 20.9 explained 29% of the IgG_2 variance on day 42. The region of the QTL contains the immunoglobulin heavy chain cluster [25,26]. Consequently, there is a high probability that variation in IgG_2 levels is due to allelic variation directly within a gene coding for IgG_2 antibodies (*cis*-acting). The power of the QTL was correlated with a relatively high heritability for this trait (0.42 [12]). IgG_2 antibodies on day 42 were further controlled by a QTL that mapped to the interval Sw1031–Sw2431 on SSC17. This QTL explained another 23.4% of the IgG_2 phenotypic variance on day 42, with an F value of 15.6 ($p \leq 0.001$). Both QTLs together explained 52.4% of the IgG_2 phenotypic variance.

The QTL on SSCX was associated with IgG_2 variation on day 28 ($p < 0.001$). The possible function of this QTL remains unclear. Neubert et al. [27] described a mutation in the IL2 receptor γ-chain linked to severe combined immunodeficiency, which mapped to the homologous area of the human X chromosome. IgG_2 antibody titers were decreased by Pietrain alleles at the QTL on SSC7. They were increased by Pietrain alleles at the QTLs on SSC17 and SSCX.

From a clinical perspective, the most serious phase in porcine sarcocystosis is associated with disseminated damage of endothelial cells due to the release of masses of second-

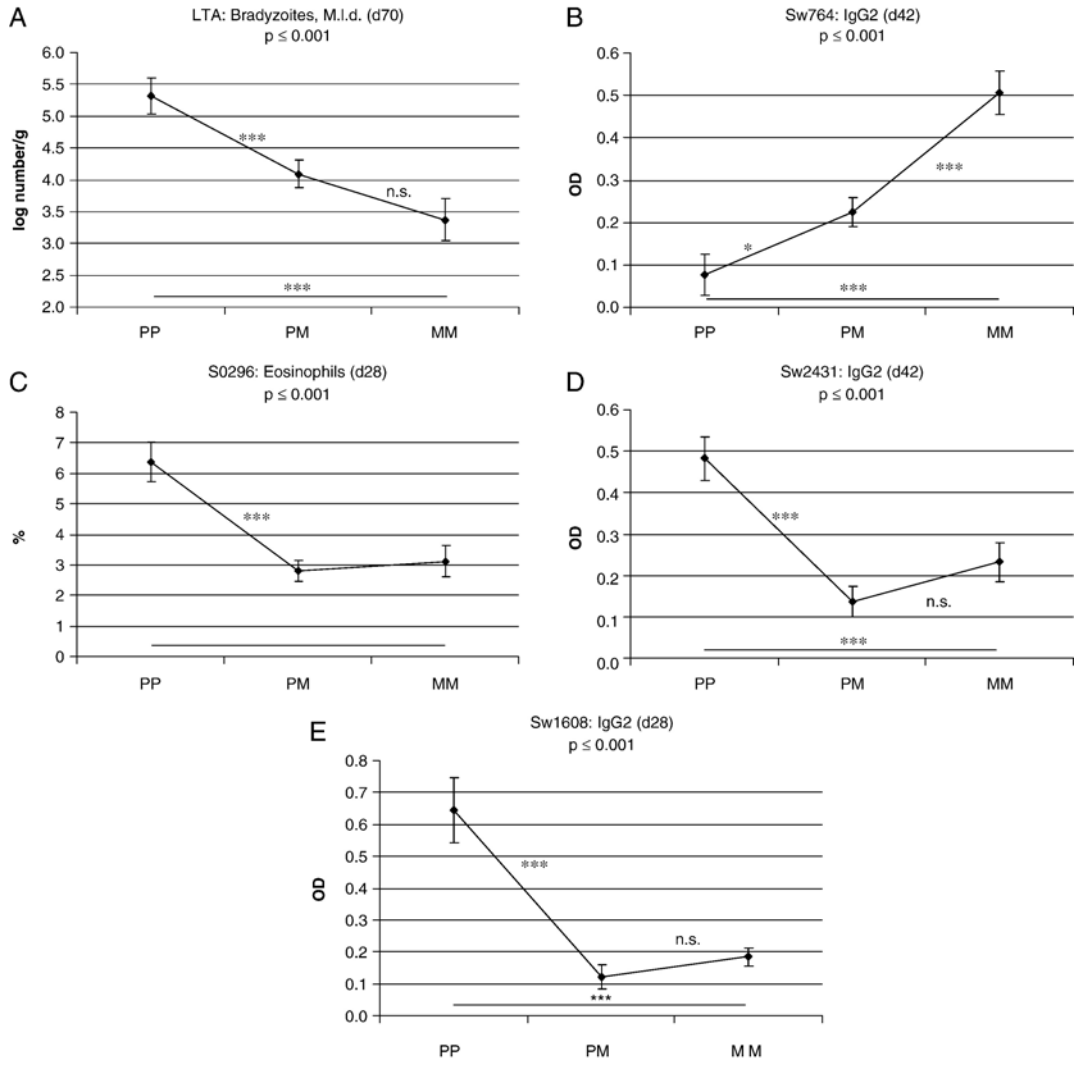

Fig. 2. Effect plots for major QTLs, based on allelic distributions determined at the peak marker of the QTLs (LTA, lymphotoxin A; Sw764, SSC7; S0296, SSC7; Sw2431, SSC17; Sw1608, SSCX). The three possible genotypes are indicated on each ordinate (P, Pietrain; M, Meishan). The *y* axis represents (A) logarithm of number of bradyzoites per gram of musculus longissimus dorsi 70 days postinfection; (B, D, and E) OD of *S. miescheriana*-specific IgG_2 antibodies at different time points p.i.; (C) percentage of eosinophil granulocytes on day 28 p.i. Error bars represent SEM. $*p \leq 0.05$; $***p \leq 0.001$; n.s., not significant.

generation merozoites. This phase is clinically characterized by a pronounced fever peak around day 14 p.i. [11,15]. An additional fever episode occurred in highly susceptible Pietrain pigs 6 weeks p.i., i.e., with early cyst formation, an episode that was absent in the resistant Meishan pigs [11]. Therefore, these fever attacks also seem to be an indicator of parasite proliferation.

Rectal temperatures showed only low to moderate heritabilities, whereby the variability was associated with a set of at least 10 QTLs, each with a small effect. Among them were 6 QTLs with effects during day 14 and 2 with effects during day 42. These QTLs explained up to 15.7% of the F_2 phenotypic variance with F values of up to 10.4. Nonetheless, in combination they explained 69 and 35% of the phenotypic variance on days 14 and 42, respectively. The most dominant QTL for body temperature mapped to a position on SSC2, which has already been identified as a QTL for body temperature in Aujeszkys disease (pseudorabiesvirus [10]). An interleukin cluster including IL3, IL4, IL5, IL9, and IL12 is located in this chromosomal area. In both cases of the *S. miescheriana* and the pseudorabies infection, the susceptible Pietrain alleles are associated with lower body temperatures. This might be interpretable as a slower interleukin-triggered immune response of the more susceptible breed.

A second QTL associated with body temperature in both infections mapped to SSC11. Again, this effect emerged during the acute phase of the *S. miescheriana* infection, with lower temperatures linked to the Pietrain alleles. A third QTL associated with the body temperature in both infections mapped to SSC16, i.e., at a chromosomal region that also seems to be involved in the control of bradyzoite numbers in the Mld. Pietrain alleles of this region were associated with higher body temperatures during acute sarcocysostis and with higher bradyzoite counts at necropsy in comparison to Meishan pigs. Nonetheless, we could not find any hints of

Table 3
Markers used for the present study by chromosome (SSC) and position

Marker	SSC	Position (cM)	Fragments (PI)	Fragments (ME)	Reference
Sw552	1	0	115 120 122	104 133	[34]
Sw1332	1	20	87 89 98	79 91	[35]
Sw1123	1	44	179 181	150 176	[36]
Sw780	1	72	115 120	130	[34]
Sw803	1	85	88 90	88 90	[34]
Sw705	1	113	143 147 156	158 161	[34]
Sw2443	2	0	208	199 210	[37]
Sw1650	2	27	122 125	141 145	[35]
Sw240	2	42	91 103	88 93 95	[34]
S0091	2	64	158 164	148 150 166	[36]
Swr2157	2	89	106 108 117 122 114	102 106 104	[35]
Swr345	2	114	136 142 159 171	139 141	[34]
Swr308	2	128	136 141 155 158	141 151	[34]
Sw72	3	0	89 93	95 107	[34]
S0206	3	25	191 199 205	175	[38]
Swr978	3	36	118	123 144	[34]
Sw2570	3	55	158 176 178 152	152 160	[39]
Sw349	3	95	152 154 173	152	[34]
Sw489	4	0	158 173	156 171 173	[34]
Sw835	4	22	220 224 228	238	[34]
S0001	4	37	181 183 187 189	177 179	[36]
S0073	4	70	85 105 108 112	95 101 108	[36]
Sw2435	4	98	142 149	155 171 180	[35]
Sw856	4	125	166 183 199	148 164 168	[35]
Sw413	5	0	162 171	160 164	[34]
Sw1482	5	32	99 134 138	81 91	[39]
Swr453	5	50	185 189	172	[34]
S0092	5	64	137 149 151 160	137 139 142	[36]
S0005	5	80	202 233 235 243	202	[36]
S0018	5	100	250 260 262	244 258	[40]
Sw378	5	126	118 120	105 115 118	[34]
S0035	6	0	174 179 182	180 183	[41]
Sw1057	6	40	155 159 166	187 191	[34]
S0087	6	56	163	193	[42]
Sw492	6	62	88 90 94 104	94	[34]
Sw824	6	104	114 132 135	114 138	[34]
Sw322	6	143	103 109 112 114	105 107	[34]
Sw2052	6	158	141 145 152	130 137 143	[39]
S0025	7	0	102 103 104	96 101	[40]
S0064	7	27	117 136 139 143	120	[36]
LTA	7	55	193 199	172 176 190	[43]
Sw2428	7	87	173	171	[45]
Sw1083	7	106	109 112	126 148	[34]
Sw581	7	120	198 204	199 204	[34]
S0212	7	137	229 234	242	[44]
Sw764	7	152	107 109 112	115	[34]
Sw2410	8	0	100 108 111	115 118	[39]
Swr1101	8	40	115 160	123 158	[34]
Sw1953	8	60	154	150 152 161	[39]
Sw2160	8	82	182 186	173 188	[39]
Sw790	8	109	168 171	179	[34]
Sw1980	8	128	183 187 195 199	187 190	[39]
Sw21	9	0	122 132 135	132 141	[39]
Sw911	9	22	155 159 166	149 155	[34]
Sw1434	9	56	138 158 160	150	[39]
Sw2093	9	89	119 131 139	96 98	[39]
Sw749	9	125	98 104	100 102	[34]
Sw767	10	0	115 122	138 150	[34]
Sw497	10	19	96 98 102	89 91 96	[34]
Sw1041	10	47	87 94	92	[34]
Sw1708	10	81	106 112 114 116 121	81 98	[39]
Sw2067	10	108	112 132 135 137	101 107	[35]
S0385	11	0	166 168 170 172	162 188	[46]

(continued on next page)

Table 3 (*continued*)

Marker	SSC	Position (cM)	Fragments (PI)	Fragments (ME)	Reference
S0182	11	33	111 127 130	127 138	[47]
Sw903	11	77	193 200 207	197 203	[34]
S0143	12	0	140 153 155 157	157 160 162	[48]
Sw957	12	27	105 122 126 135	152	[34]
Sw874	12	58	201 205 210	189 203	[34]
S0090	12	74	239 243	247	[36]
Swc23	12	90	127	130 144	[39]
Swr1021	12	107	93 102	87 91	[34]
S0219	13	0	158	172	[44]
Sw344	13	34	155 157 159 184	155 161	[34]
Sw225	13	69	86	94 102	[34]
Sw2440	13	101	136 143 153	149 151	[39]
S0215	13	120	150	132 158 192	[44]
Sw2038	14	0	104 114 118	115	[39]
Sw2519	14	32	186 215 228	195 207 222	[39]
Sw761	14	57	152 154 156	146 158	[34]
Sw2515	14	90	91 93 97	83	[37]
S0148	15	0	147 150 161 170	151 176	[48]
S0118	15	25	214	205 218	[49]
Sw2083	15	46	143 156 158	150	[37]
Sw1983	15	66	177 185 180 188 192	162 166 177	[39]
Sw742	16	0	202 204 219 223	191	[34]
Sw419	16	15	161 164 167	152 167	[34]
Sw1809	16	28	120 124	140 153	[39]
Swr2480	16	46	225 230 248	227 256	[39]
Sw1897	16	77	154 160	162	[39]
Sw335	17	0	104	93 95	[34]
S0296	17	32	164 175	179	[50]
Sw1031	17	63	86	96 104 108	[34]
Sw2431	17	94	155 161	158	[39]
Sw1808	18	0	100 149 151	133 136	[39]
Sw787	18	32	152 154 160	152 156	[34]
S0120	18	46	151 170	161 166	[49]
Sw949	X	0	185 205	172 185	[34]
Sw2126	X	36	141 148	153 155	[39]
S0117	X	75	160 162	181 185	[49]
Sw1608	X	103	119 135	121	[39]

candidate genes for this region. Thus body temperature seems to be controlled by many QTLs, each with only small effects.

In regard to clinical–chemical traits, Pietrain and Meishan pigs had significantly different serum AST and CK levels before and during the *S. miescheriana* infection [11]. Both traits showed clear additive genetic inheritance [12]. Nine putative and four genome-wide significant QTLs were mapped for AST and CK plasma levels.

Sixteen putative QTLs and 3 QTLs significant on a genome-wide level were mapped for the number of eosinophils and platelets. As with the clinical traits, the relatively high number of QTLs might reflect an explicit polygenic character of these traits. Potential homologous candidate genes were hardly detectable in the areas of these QTLs. More detailed studies will be needed to resolve the basic mechanisms of regulation and interaction leading to these phenotypic effects.

In conclusion, major QTLs for the traits studied in the course of acute and chronic sarcocystosis suggest genetically based differences in resistance/susceptibility to the disease in swine [10,11]. Especially the QTLs for bradyzoite numbers and IgG_2 titers on SSC7 explain phenotypic variance ratios comparable to extraordinary QTLs for performance traits already in use in pig breeding programs (e.g., IGF2 [28]). The positional associations of some of the QTLs with physiological candidate genes (e.g., bradyzoites and MHC/TNF, IgG_2, and Ig heavy chain cluster) provide some promising candidate genes, which should lead to an elucidation of the molecular basis of resistance/susceptibility of pigs to *S. miescheriana*.

There is a relatively large number of QTLs with large effects involved in the variability of resistance to *S. miescheriana*. This reflects the high complexity of this host–parasite interaction, affecting a broad scope of clinical, clinical–chemical, immunological, and parasitological traits. Because the parasite used in this study is exemplary of many parasites, the results of the present study might have a valuable impact on the understanding of other host–parasite interactions. It might be interesting to carry out comparative studies on other protozoan infections. Establishing the responsible genes would help to improve our knowledge of host–parasite interactions and host defense mechanisms.

Materials and methods

Experimental pigs

The experiments were based on a Pietrain/Meishan family reared at the experimental station "Unterer Lindenhof," University of Hohenheim, Stuttgart, Germany. Four Meishan sows and 4 Pietrain boars served as founders of the family. Twelve F_1 sows and 5 F_1 boars produced 18 litters from which 139 F_2 pigs were consecutively included into the study. All these pigs were serologically negative for antibodies to *S. miescheriana* as determined by ELISA and clinically healthy at the start of the experiment. The pigs were free of helminths or other parasitic infections.

Three weeks before infection, the pigs were transported to Giessen, Germany, and allowed to acclimatize. Groups of 5 to 11 pigs per pen (altogether 11 study groups) were housed in tiled boxes of 16 m^2 on straw bedding. Starting with the date of arrival, they received antibacterial food medication for 6 days (200 ppm tiamulin and 1000 ppm chlortetracycline). Subsequently, the pigs were fed a customary cereal diet (energy content: 11.5 MJ/kg) without food additives ad libitum. The pigs were accustomed to human interaction by daily handling over a period of 2 weeks. Each pig was monitored daily throughout the week before infection, to serve as its own control.

Infection and clinical examination

S. miescheriana sporocysts were collected from the intestine of a dog fed with cyst-harboring pig meat as described in detail by Rommel et al. [29]. Sporocysts were stored at 4°C until used. The pigs were infected orally at an age of approximately 100 days (day 0) with 50,000 sporocysts per animal.

The clinical status of the pigs, including rectal temperature and general symptoms of disease, was examined daily from day 7 before infection (day −7) to the day of infection (day 0) and on days 7, 12, 13, 14, 21, 28, 35, 42, 45, 49, 56, and 63 p.i. Blood samples were drawn on days 0, 14, 28, and 42 p.i. from the cranial vena cava. The pigs were slaughtered on day 70 p.i.

Clinical–chemical examinations

Blood cell counts were determined from EDTA blood samples using an automatic cell counter (Sysmex, F-800, Langenfeld, Germany). Leukocytes were differentiated in Papenheim-stained thin blood films (Leukodiff 800, Instrumentation Laboratories, Kirchheim, Germany). The activities of AST and CK in the plasma were measured by customary spectrophotometric tests.

Antibody determination

Sarcocystis-specific antibody titers were determined by ELISA. An antigen was prepared from a muscle sample obtained from a pig slaughtered 70 days after infection with 50,000 sporocysts of *S. miescheriana*, according to Reiner et al. [12]. *S. miescheriana*-specific IgM, IgG, IgG_1, and IgG_2 antibodies were determined by ELISA as described by Reiner et al. [12]. In brief, microtiter plates (Nunc Maxisorp) were coated with 3 and 1 μg of antigen in 0.1 M carbonate buffer (pH 9.6) per well for IgG and IgM antibody determination, respectively. One hundred microliters of diluted serum samples (1:300 in 1% BSA/PBS) was added to each well and incubated for 1.5 h at 37°C. The plates were incubated with 100 μl of horseradish peroxidase-conjugated anti-porcine IgG or IgM antibodies (Serotec) per well, diluted 1:50,000 with 1% BSA/PBS. OD was measured with a microplate reader.

S. miescheriana-specific IgG1 and IgG2 antibodies were determined by a double sandwich ELISA, using 0.75 and 1.5 μg antigen/well, respectively. Procedures were the same as above, except that the mouse anti-porcine IgG1 (1:500) or the anti-porcine IgG2 (1:300; both Biozol) were used as first conjugates, followed by anti-mouse IgG–horseradish peroxidase-coupled antibodies (Biozol) diluted 1:1000 for IgG1 and 1:500 for IgG2 as second conjugates.

Parasite burden

Parasite burden was determined in the cardiac muscle and the Mld. Twelve grams of the tissues was subjected to tryptic digestion for 45 min at 37°C, according to the method described by Jäkel et al. [30]. Released bradyzoites were counted in a Neubauer chamber.

Statistical analysis

Data were analyzed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). Bradyzoite counts and CK values were logarithmized to obtain an approximately normal distribution. Values of traits obtained on days 12, 13, and 14 were used to characterize the acute stage of the infection. The values obtained on day 28 were regarded as representing the phase of recovery from the acute stage. The data of day 42 were used to describe the chronic (cyst-forming) stage of the infection.

Markers

Marker loci were selected to be amply informative and evenly distributed over the chromosomes. Of 150 reported microsatellites, 101 fulfilled these criteria (Table 3) and were genotyped in the F_2 family. The average distance between markers was 25.2 cM.

QTL analysis

QTL analysis was performed using the Web-based application "QTL Express" [31]. Analysis was based on a least-squares method developed for crosses between inbred and outbred lines [32]. The general linear model was preadjusted for the fixed effects of sex and study group. The age at infection was included as a covariate. Chromosome-wide and genome-wide significance levels were estimated by permutation tests [33].

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